Vision

Sunday, October 16, 2016 1:24 PM

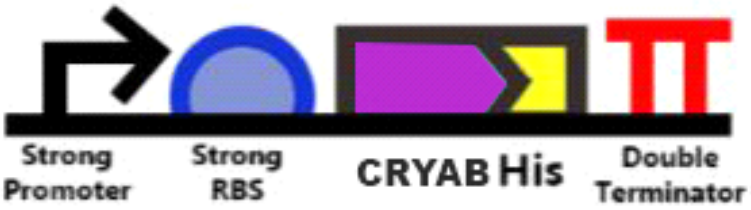
We envision maximizing the desired proteins so that lots of proteins can be purified for use per single E.coli colony.

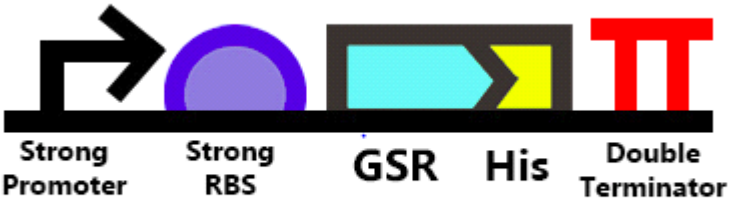
To maximizing the proteins , we decided on using strong constitutive promoter and strong RBS, **BBa_K880005** obtained from iGEM Distribution Kit.

To purify the proteins completely from the bacteria, we used 10x Histidine Tag **BBa_K884000.**

To end the transcription we cloned in double terminator **B0015** C-terminus.

The final composite part for our projects will look as illustrated below:





BBa_K880005 plasmid cDNA Basic Information

Sunday, February 28, 2016 3:01 PM

Part Size	55bp
Antibiotic Resistant	Chloramphenicol
Primers for sequencing	Forward: VF2 Reverse: VR
Reference Sequence	
Storage	Lab -20C Fridge (Counteracts Box) along with its **primers**

Bba_K880005 cDNA Transformation

Wednesday, February 24, 2016 2:58 PM

Goal

- To insert Bba_K880005 into bacterial cells so that the cDNA can be infinitely reproduced.

Procedure

- **BBa_K880005** (from distribution kit 2015, **3F**): reconstituted with 10ul water
- Transformation (new protocol: non-heat shock)
  - Thaw competent cell vial (100ul total) --- transfer 50ul to clean new tube (**Fire On**)
  - Add DNA (less than 10% of volume of cells). Vortex 1 second (**Fire On**)
    - Add 1ul DNA
  - Place on ice for 10min
  - Plate all transformation mix on LB + Cm using glass beads (**Fire On**)
  - Incubate overnight at 37C

BBa_K880005 PCR Check

Friday, February 26, 2016 3:10 PM

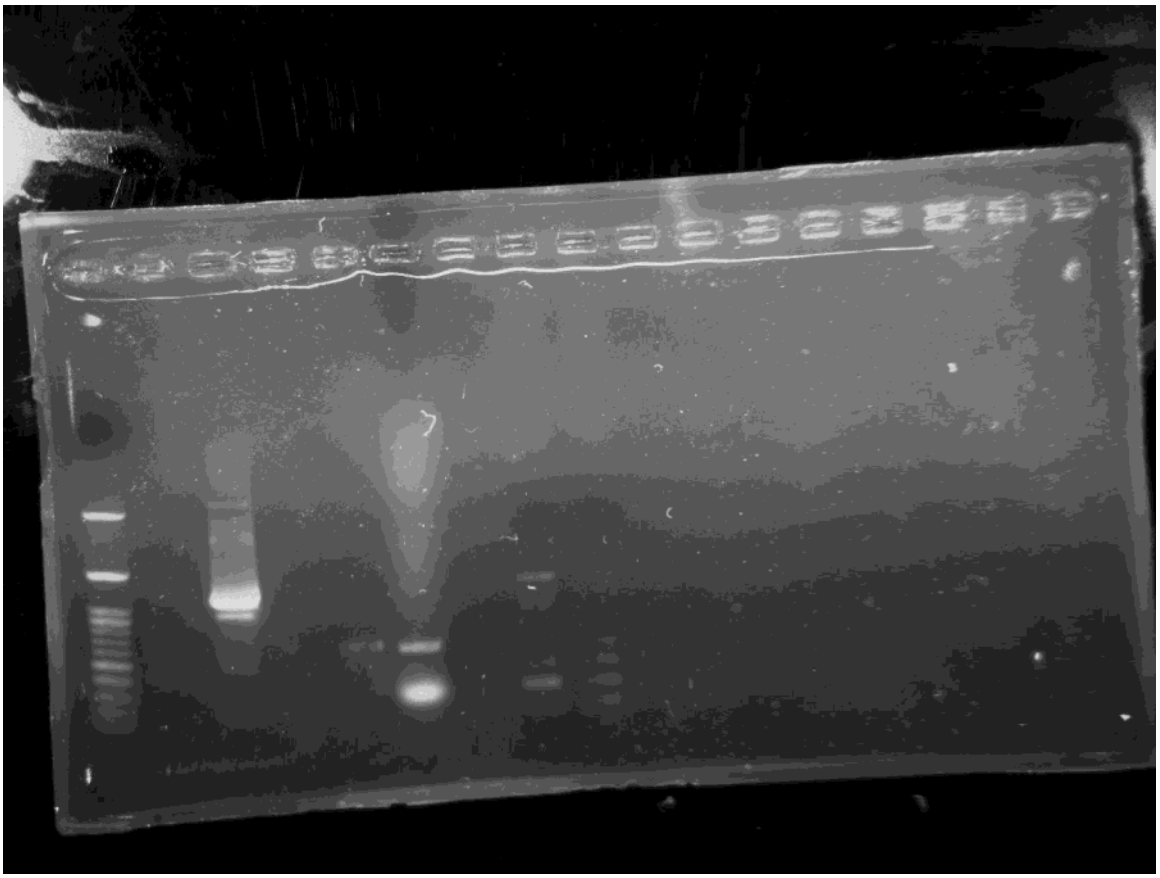
Goal
To certify that the cDNA is properly expressed in the cell.

Procedure

*2x Taq PCR Check
*Before putting in 1 bacterial colony, the colony was streaked on another plate to continue the cultivation of the Bba_880005 bacteria

BBa_K880005 Gel Check & Liquid Culture

Monday, February 29, 2016 8:14 PM



Lane	1	2	3	4	5	6	7	8	9
Substance	100bp DNA Ladder		GFP Gene (Control)		CRYAA (Restreak 1)	CRYAA(Restreak 2)		Bba_K880005 (Restreak 1)	Bba_K880005 (Restreak 2)
Band Size	N/A		~1kbp		~822bp	~822bp		~355bp	~355bp

BBa_K880005 Miniprep

Thursday, March 03, 2016 10:44 AM

Goal: To obtain pure plasmid of Bba_K880005 in igem backbone

Procedure

Gene aid Miniprep kit protocol

Result

Concentrations

RESTREAK 1 BBa_K880005 1: 615.7 ng/ul

RESTREAK 2 BBa_K880005 1: 320.8 ng/ul

RESTREAK 2 BBa_K880005 2: 443.1 ng/ul

RESTREAK 2 BBa_K880005 3: 441.7 ng/ul

Conclusion

Bba_K880005 is ready to be used for cloning in IGEM backbone!!!

BBa_K880005 Digestion and Gel Run

Wednesday, March 9, 2016 3:38 PM

<u>Goal:</u> To cut out the s,p region so that GFP + 10x His-Tag can be inserted to make a active 10x His-Tag Bio Brick

<u>Same Protocol As Cloning Cycle</u>

Size

- BBa_K880005: 55bp
- Vector: 2000bp

Concentration

- 615.7

Volume (uL)

	DNA	Water
BBa_K880005	1.62	14.38

Cutting sites:

- S&P

<u>Goal:</u> To cut out the vector so that GFP + 10x His-Tag can be ligated into the vector to complete the full bio brick

BBa_K880005 Backbone (Vector) Gel Run and Purification

Wednesday, March 9, 2016 5:29 PM

Goal To separate out the cut vector BB_k88005 from the gel and purify it

Procedure

Ran gel for BBa_K880005 Backbone (Vector_

Write down Voltage, and time taken to run the gel

Write down the protocol for gel extraction or just say "same as that of cloning cycle"

Result

Post Gel pic

And the concentration of the purified DNA:

Conclusion

Stored in 4c Igem exp. In progress box

Bba_K880005 Miniprep/Restock

Tuesday, March 22, 2016 5:11 PM

Goal: To restock Bba_K880005 pure plasmid to use it for later experiments

Procedure:

Same as Cloning Cycle Miniprep

Result:

Concentration: 367.4 ng/ul

Conclusion:

Next Step -> To be determined

BBa_K844000 (10x Histag) plasmid cDNA information

Tuesday, June 21, 2016 10:06 AM

Part Size	36bp
Antibiotic Resistant	Chloramphenicol
Primers for sequencing	Forward: VF2 Reverse: VR
Reference Sequence	
Storage	Lab -20C Fridge (Counteracts Box) along with its **primers**

10xHis-tag cDNA Transformation

Wednesday, February 24, 2016 3:03 PM

Goal: To transform 10x His-tag_TT into competent cells.

- **Bba_K844000** (from distribution kit 2015, **5P**): reconstituted with 10ul water
- Transformation (new protocol: non-heat shock)
    - Thaw competent cell vial (100ul total) --- transfer 50ul to clean new tube (**Fire on**)
    - Add cDNA (less than 10% of volume of cells). Vortex 1 second (**Fire on**)
        - Add 5ul cDNA
    - Place on ice for 10min
    - Plate all transformation mix on LB + Cm using glass beads (**Fire on**)
    - Incubate overnight at 37 degrees Celsius

10xHis-tag PCR check

Wednesday, February 24, 2016 3:30 PM

10xHis-tag gel check after PCR

Wednesday, February 24, 2016 3:30 PM

Goal:
To check whether the PCR of cDNA of 10x His-tag _ TT was successful

10x Histidine Tag _ TT

Part Sequence Length: 36bp

Product Length after PCR (including Prefix and Suffix) : 336bp

GFP Gene _ TT (**Control**)

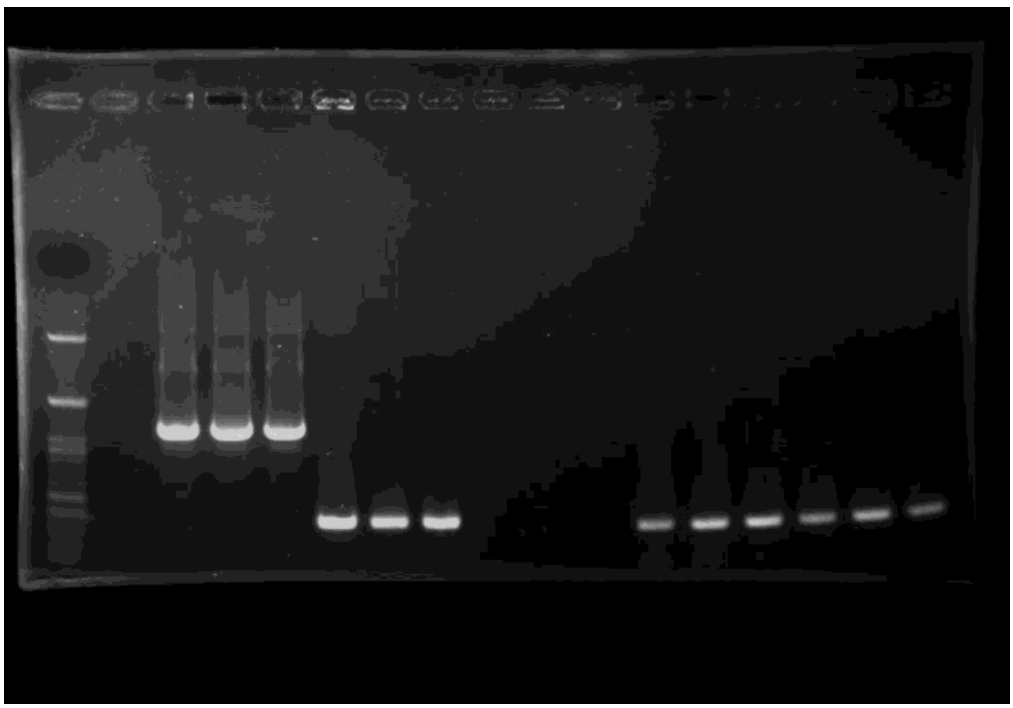
Part Sequence Length:

Product Length after PCR (including Prefix and Suffix) :

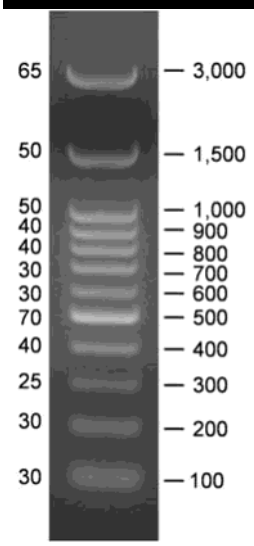
Procedure

- Put in 20ul of DNA Ladder (100bp) in the very left well in the 17 well gel
- Load 20ul (total) of each 9 (numbered by colonies from the transformation) PCR mix in a separate well
  - **First three – Controls**
  - **Rest – PCR Mix (9 colonies)**
- Run Gel electrophoresis
  - 110 Volt
  - 20 minutes
- Place the gel under UV and capture screen

Result



[redacted]



GFP Gene is approximately around 900bp (the value we predicted)

- Shows how the gel run is successful

10x Histidine Tag is approximately around 300bp

- Given that we know the gel run is successful because of the control (GFP Gene), we can trust the gel result for 10x Histidine Tag

10xHis-tag liquid cultures

Wednesday, February 24, 2016 3:32 PM

10xHis-tag Miniprep

Wednesday, February 24, 2016 3:33 PM

Same Protocol as (Cloning Cycle)

Concentration of miniprep solution

His-tag 1: 143.7 ng/ul
His-tag 2: 144.3 ng/ul
His-tag 3: 168.3 ng/ul
His-tag 4: 165.6 ng/ul

Conclusion:

10x His tag pure plasmid can now be used for cloning constructs.

Bba_K880005_GSR_His_term

Tuesday, October 18, 2016 6:43 PM

GSR cDNA Basic Information

2016年2月28日　　下午 03:10

ORF Size	1569bp
Antibiotic Resistant	Ampicillin
Primers	Designed
Reference Sequence	NM_000637.2
Storage	Lab -20C Fridge (Counteracts Box) along with its **primers**

Further Information

http://www.origene.com/human_cdna/NM_000637/SC320532/GSR.aspx

GSR cDNA Transformation

Wednesday, February 24, 2016 1:51 PM

Goal

- To insert CRYAA into bacterial cells so that the cDNA can be infinitely reproduced.

Procedure

- GSR cDNA (from Ori gene)-- 10mg DNA reconstituted with 100ul water: 100ng/ul
- Transformation (old cells)
  - Thaw competent cell vial (100ul total) --- transfer 50ul to clean new tube (**Fire on**)
  - Add DNA (less than 10% of volume of cells). (**Fire on**)
    - Add 1ul DNA (@100 ng/ul)
  - Place on ice for 5 mins
  - Heat shock at 42 C for 30 seconds
  - Place on ice for 10 mins (**Fire on**)
  - Plate all transformation mix on LB + amp
  - Incubate overnight at 37 degrees Celsius

Conclusion:

Pending for PCR Check to determine whether E.coli successfully expresses GSR.

GSR cDNA PCR Check(postponed)

Friday, February 26, 2016 3:12 PM

Ampicillin Gels Ran out for RESTREAK – **Postponed**

GSR cDNA PCR Check & Gel Check

Monday, February 29, 2016 8:11 PM

Goal

- To certify that the cDNA is properly expressed in the cell.

Procedure

- Ran 2x Taq PCR and ran gel
- Before putting in 1 bacterial colony, the colony was streaked on another plate to continue the cultivation of the GSR bacteria

Results

- Accidentally used wrong primers (VF2, VR). Need to be redone
- Hence the original Gel Check Failed
    - No Bands were shown

GSR cDNA PCR Check 2

Wednesday, March 2, 2016 2:53 PM

Goal

- To reconfirm that the cDNA is properly expressed in the cell.

Procedure

- Ran 2x Taq Polymerase
- No need for restreak, since the plate was restreaked the day before while doing the initial PCR check which failed because of using wrong primers
- Used a colony from the Restreak plate

Conclusion

Must carry out gel check.

GSR Miniprep & Digestion

Friday, March 04, 2016 12:15 PM

Goal: To miniprep and digest the pure plasmid and check the bands through gel to see if GSR cDNA was correctly transformed. (alternative solution to Gel check)

Procedure

Geneaid Miniprep Kit Protocol,

Result

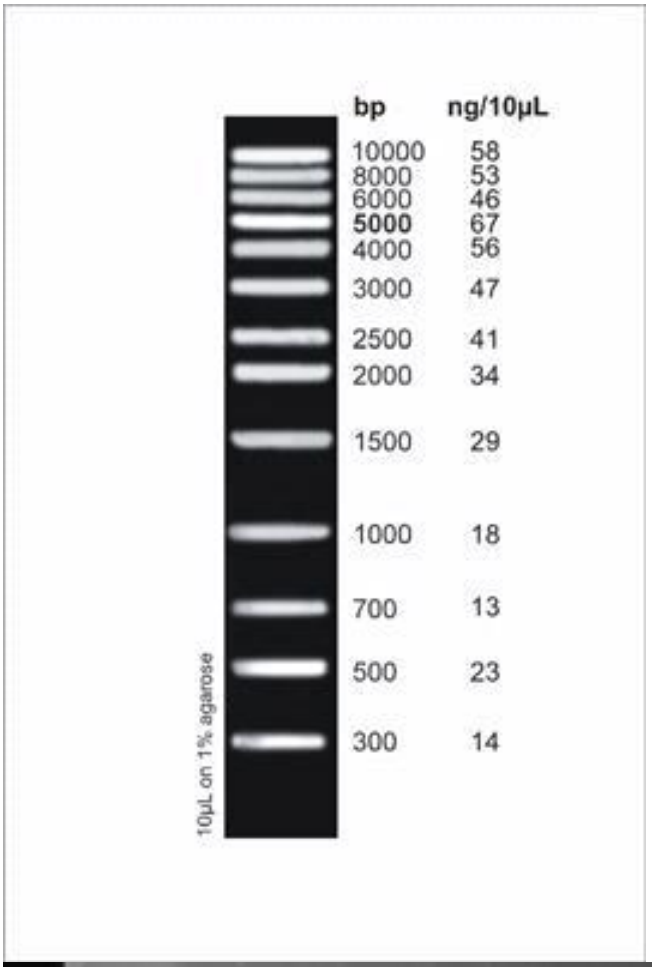
Concentration

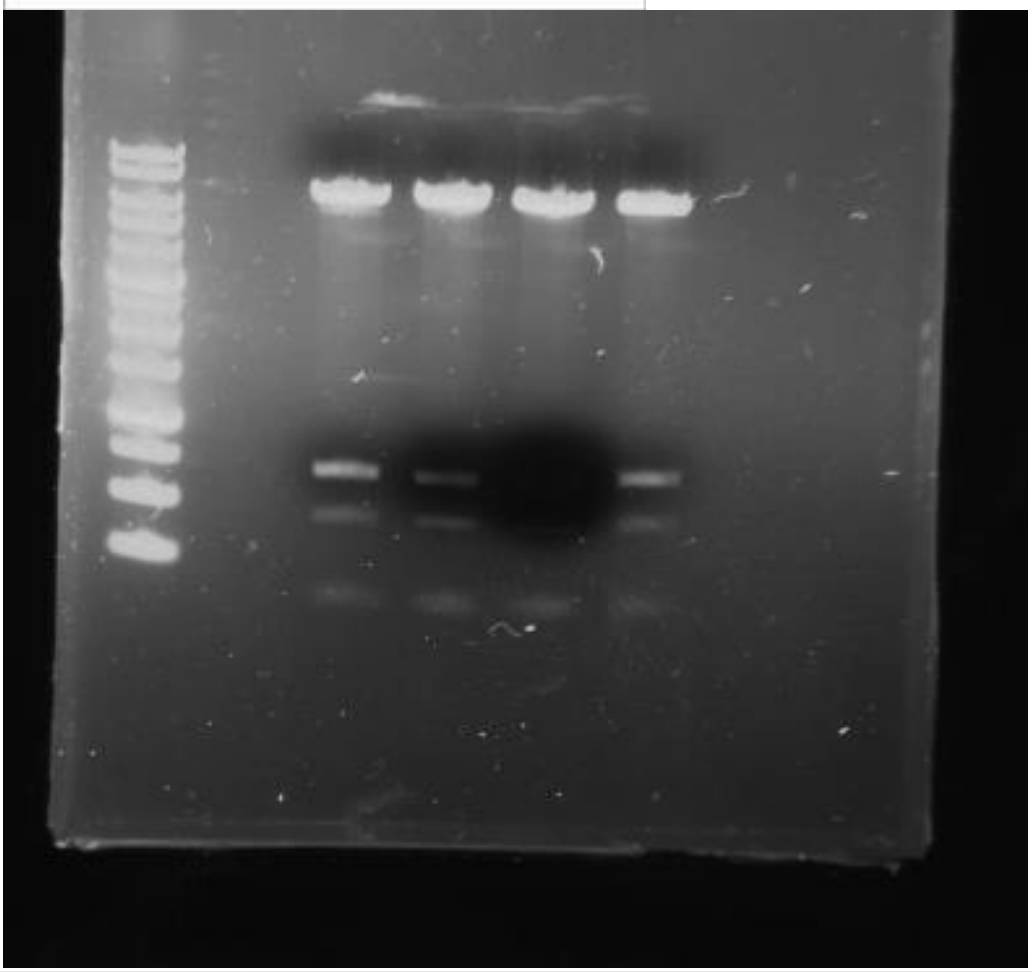
RESTREAK 1 BBa_K880005 1: 615.7 ng/ul

RESTREAK 2 BBa_K880005 1: 320.8 ng/ul

RESTREAK 3 BBa_K880005 2: 443.1 ng/ul

RESTREAK 4 BBa_K880005 3: 441.7 ng/ul


bp
ng/10µL
10000 58
8000 53
6000 46
5000 67
4000 56
3000 47
2500 41
2000 34
1500 29
1000 18
700 13
500 23
300 14
10µL on 1% agarose



Lane	1	2	3	4	5	6	7	8	9
Substance	1kbp DNA		GSR 1 Digestion	GSR 2 Digestion	GSR 3 Digestion Mix	GSR 4 Digestion Mix			
Band Size	N/A		6.3kb 700bp 360bp	6.3kb 700bp 360bp	6.3kb 700bp 360bp	6.3kb 700bp 360bp			

Result

Cut bands give inconclusive result

GSR Sent out for sequencing...

GSR PCR Run + Purification

Thursday, April 14, 2016 5:38 PM

Goal
To run PCR with GSR and its primers and attach the restriction sites (+300bp)

Purify GSR (restriction sites added) DNA

Procedure

There was only 20ul of PCR Mix so only switch 100ul in the 1) step with 20ul but keep the rest of the protocol the same

1. Transfer 100 µl (pcr mix) to 1.5 ml microcentrifuge tube
2. Add 5X volume (of pcr mix) Gel/PCR buffer
3. Vortex
4. If turned yellow add 10µl 3M Sodium Acetate (in the kit)
5. Transfer sample into 2ml collection tube and DFH column
6. Centrifuge 14-16,000 xg for 30sec
7. Discard flow through, place column back into centrifuge tube
8. Add 600µl wash buffer (w/ ethanol)
9. Let sit for 1 min
10. Centrifuge 14-16,000 xg for 30 sec
11. Discard flow through
12. Centrifuge again for 3 min
13. Transfer into 1.5ml centrifuge tube
14. Add 20-50µl elution buffer to center
15. Let sit for at least 2 min
16. Centrifuge 14-16,000 xg for 2 min
17. DONE!

Results
- [sample 1] =TBD ng/µl

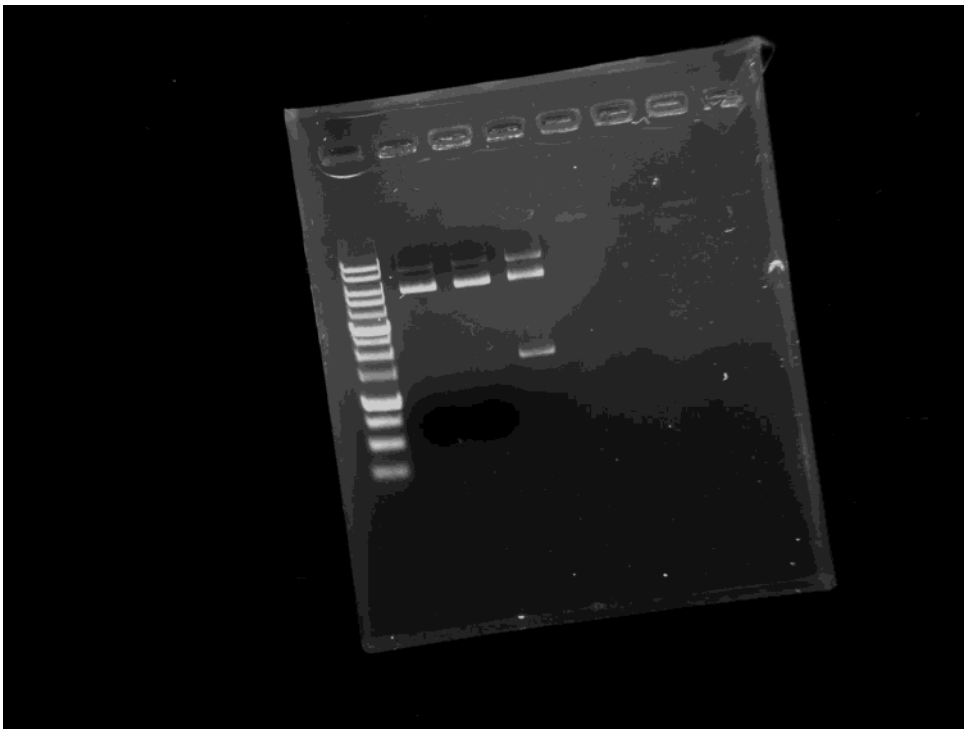
Conclusion
- Needs to be digested and put into an igem biobrick

GSR w/Restriction Sites Gel Check

Monday, May 2, 2016 4:06 PM

Goal: To check whether the restriction sites were correctly added to GSR.

Result:



Lane 1	Lane 2	Lane 3	Lane 4
1kb Ladder	GSR (~ 1.8kb)	GSR (~1.8kb)	GSR (~1.8kb)

Conclusion:

Only the GSR part in Lane 4 was amplified

GSR w/o Restriction sites Liquid Culture & Digestion of Unlabeled GSR Pure Plasmid

Wednesday, May 18, 2016 7:58 AM

Goal : To make liquid culture from Plates containing Commercial GSR

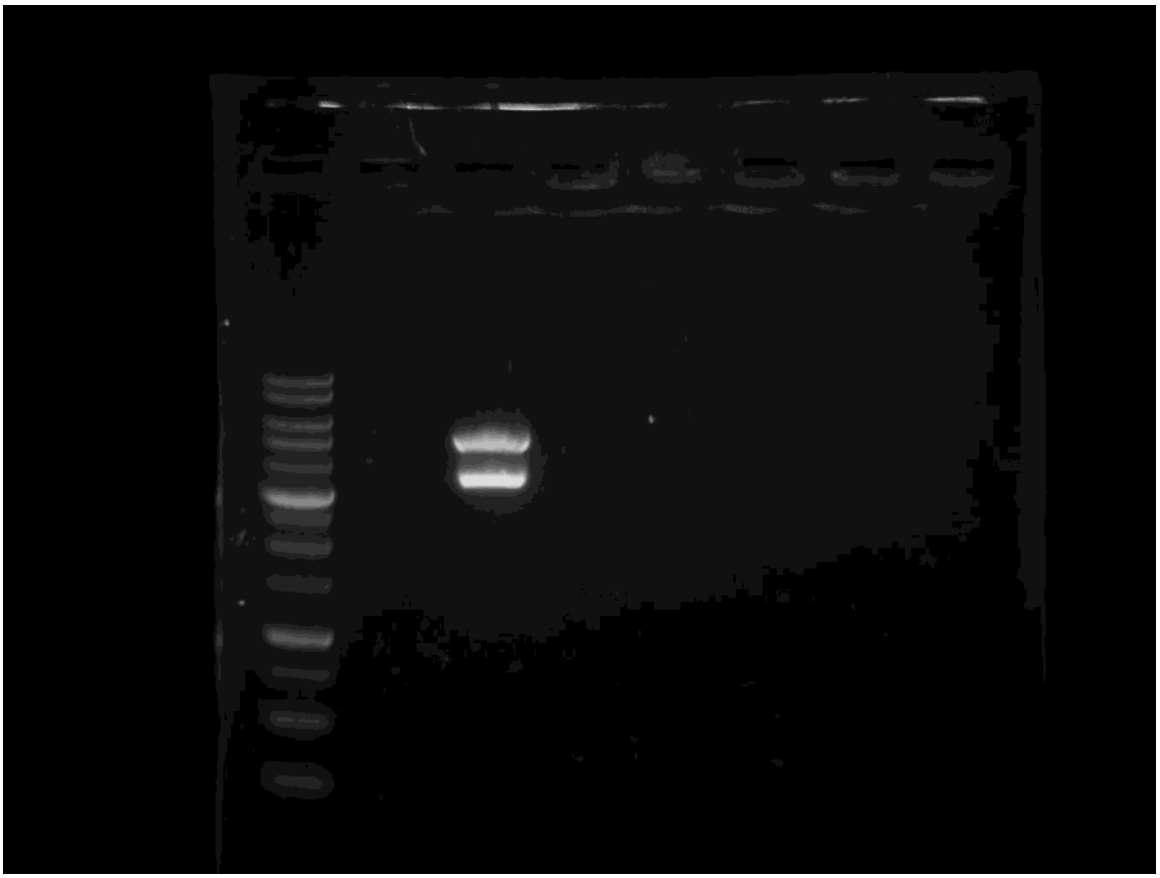
Procedure: Same as ever

Goal: The plate containing GSR with Restriction sites seems to be lost. We should check whether the GSR Pure Plasmid is in commercial vector or in iGEM backbone.

Procedure:

Digested the pure plasmid. If the length of the vector exceeds 4kb it is that of commercial vector and if it is around 2kb it is that of iGEM backbone

Result:



Conclusion:

The vector is around 4-5kb ladder mark. The GSR is in commercial vector and the plasmid does not have its restriction sites added.

Next Step -> GSR PCR Phusion

GSR w/o Restriction sites GSR Miniprep

Friday, May 20, 2016 2:08 PM

Goal : To make pure plasmids of GSR to run PCR Phusion

Procedure: Same as ever

Result:

Concentration

GSR (Restreak 3) : 1187ng/ul

Conclusion

Concentration abnormal, Previous GSR plasmid that was used for digestion is used instead for PCR Phusion

GSR w/o Restriction Sites PCR Phusion

Friday, May 20, 2016 2:14 PM

Goal: To add restriction sites to Commercial GSR

Procedure:

Based on Thermo - Phusion PCR 2 step Protocol with 20ul Rxn mix

GSR w/o Restriction Sites PCR Phusion Re-do

Tuesday, May 24, 2016 3:09 PM

GSR w/ Restriction sites PCR Clean Up and Bba_GFP_Term Digestion, Gel extraction

Thursday, May 26, 2016 2:26 PM

Goal: Replace Bba_GFP_Term construct with GSR 5 mut w/ Restriction sites so GSR has an IGEM Backbone

Procedure:
Same as "Cloning Cycle" Digestion

Result:
GSR Concentration : 3.1 ng/ul
Vector Concentration: 7.0 ng/ul

Conclusion:

Next Step -> Ligation and Transformation

GSR w/ Restriction sites Ligation and Transformation into iGEM backbone

Thursday, May 26, 2016 2:25 PM

GSR w/ Restriction sites in iGEM backbone PCR Check

Monday, June 06, 2016 11:46 AM

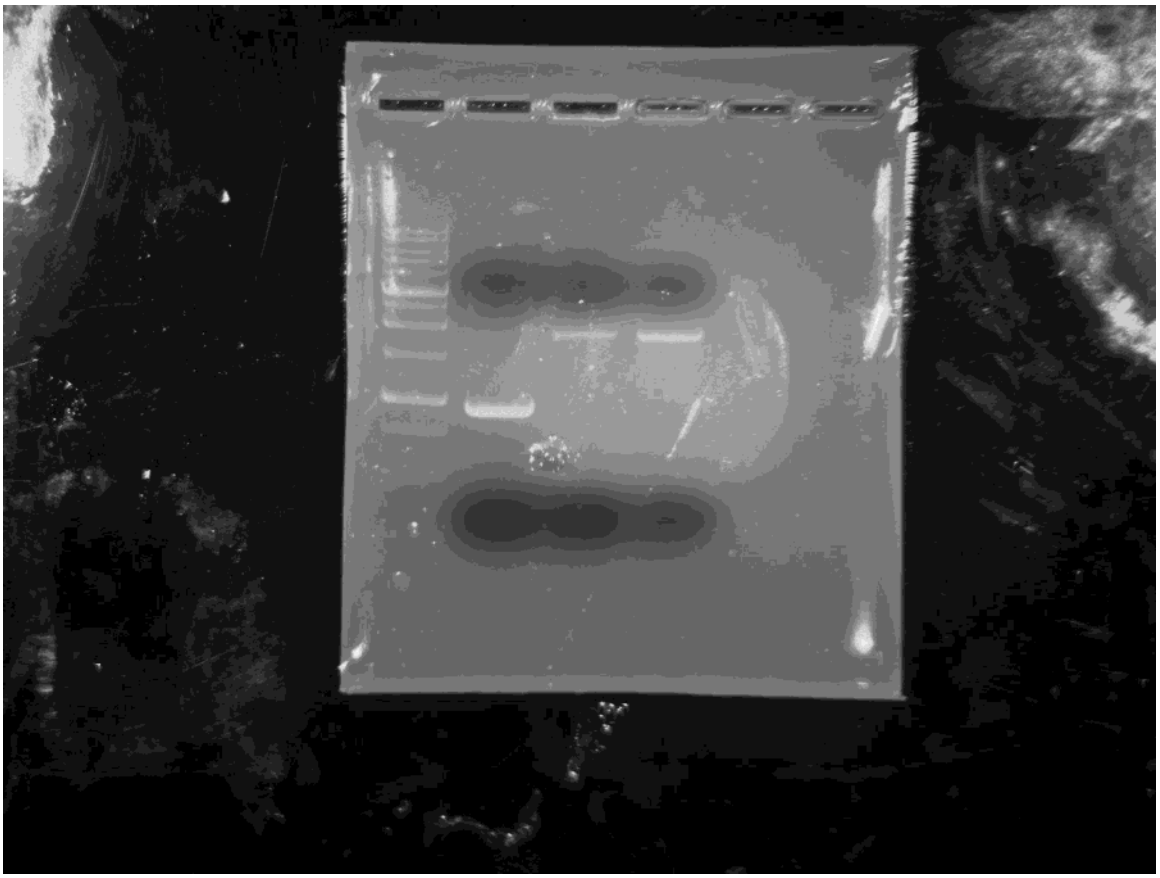
GSR w/ Restriction sites in iGEM backbone Gel Check

Monday, June 06, 2016 12:54 PM

Goal: To check whether one of the colonies of GSR-igem vector transformation clearly expresses GSR

Procedure: //

Result:



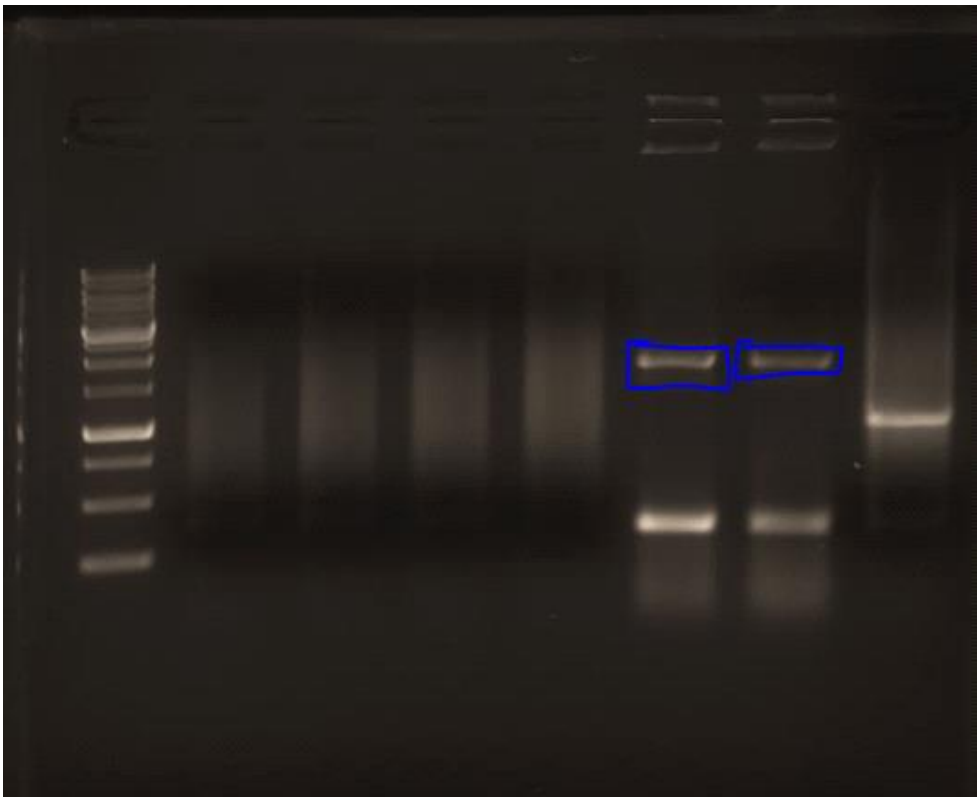
Ladder	GFP	GSR Colony -6	GSR Colony - 6
1kb Ladder	~1000kb	~2000kb	2000kb

Conclusion:

Colony 6 of GSR in igem backbone showed correct band so GSR is successfully transformed

GSR w/ Restriction sites in iGEM backbone Gel Check 2

2016年6月10日　　上午 11:55



Lanes:
1: 1 kb ladder
2: amp CH25H 1 [gen 3]
3: amp CH25H 2 [gen 3]
4: amp CH25H 3 [gen 3]
5: amp CH25H 4 [gen 3]
6: GSR 1
7: GSR 2
8: amp CH25H 8

GSR (from now on with restriction sites in iGEM backbone) Liquid Culture

Monday, June 06, 2016 1:05 PM

Goal: To get pure plasmid of GSR in iGEM Backbone

Procedure: Same as Ever

Result:

16 hours in 37C shaker (230rpm)

Conclusion:

Next Step-> Miniprep

GSR Miniprep

Monday, June 06, 2016 10:21 PM

GSR plate lost...

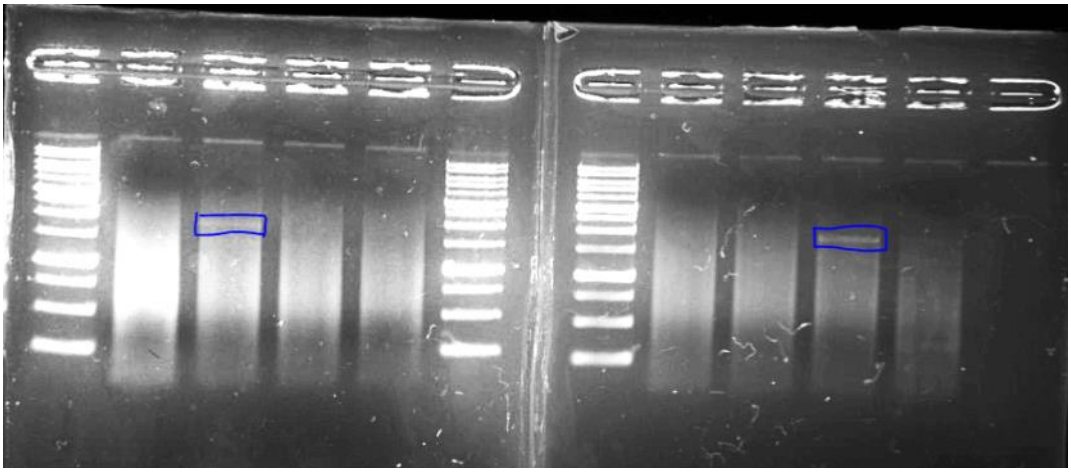
2016年6月16日　　下午 04:28

Transformed existing GSR DNA that was miniprepped

2016年6月16日　　下午 04:47

GSR (in iGEM Vector) PCR Check Redo – Success

2016年6月14日　　下午 04:51



Gel 1
Lane:

1. 1 Kb ladder
2. GSR+Vector Colony 1 (C1)
3. C2
4. C3
5. C4
6. C5

Gel 2
Lane:

1. 1 Kb Ladder
2. C6
3. C7
4. C8

GSR Miniprep & Digestion, Gel extraction, Ligation and Transformation w/ BBa_K880005

2016年6月16日　　下午 04:50

Goal: To back insert GSR into BBa_K880005 and transform the plasmid into bacteria

Procedure: Cloning Cycle

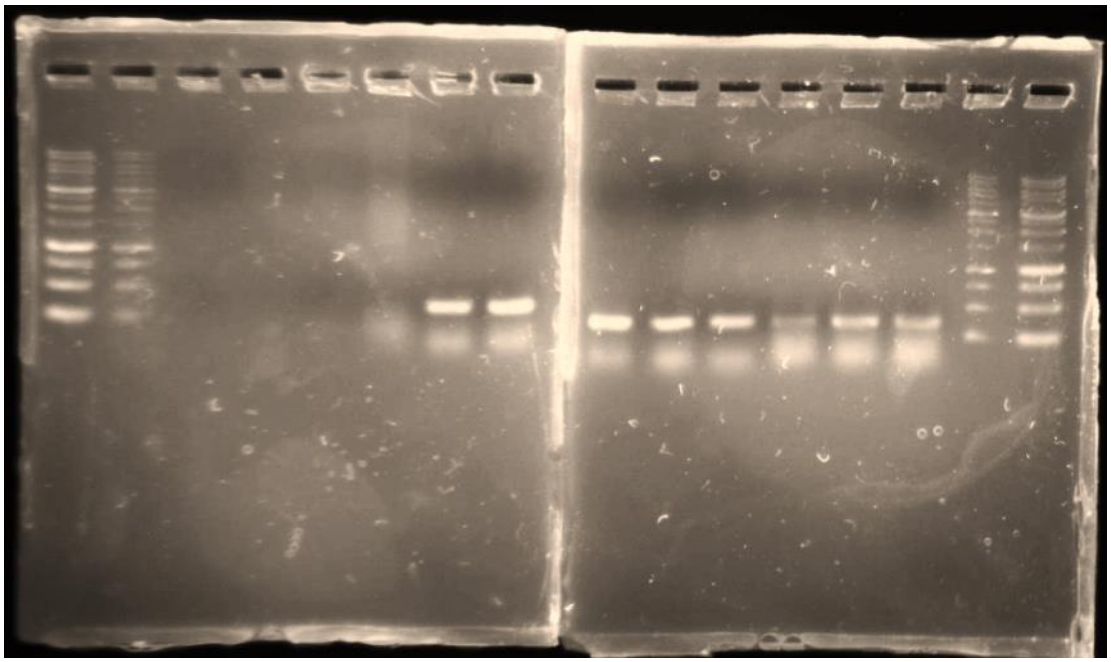
Bba_K880005_GSR 3 in1

2016年6月16日　　下午 04:53

Goal: To check if GSR is correctly ligated into BBa_K880005 and harvest cells for miniprep if it is correctly ligated

Procedure: Same as cloning cycle

Result:



Left Gel:
1) 1000 bps DNA Ladder (Dye)
2) 1000 bps DNA Ladder (Clear)
3) Bba_CH25H_His_Term – 1
4) Bba_CH25H_His_Term – 2
5) Bba_CH25H_His_Term – 3
6) Bba_CH25H_His_Term – 4
7) Bba_GSR C1
8) Bba_GSR C2

Right Gel:
1) Bba_GSR C3
2) Bba_GSR C4
3) Bba_GSR C5
4) Bba_GSR C6
5) Bba_GSR C7
6) Bba_GSR C8
7) 1000 bps DNA Ladder (Clear)
8) 1000 bps DNA Ladder (Dye)

Conclusion:
All of Bba_GSR are incorrect

Cloning Cycle Redo

Wednesday, June 29, 2016 12:19 PM

All the previous cloning cycle including Bba_K880005 and His Tag Term were all shut down because the sequencing revealed that we did not have both part.

GSR in iGEM Backbone/Vector was correct.

August 11th Bba_K880005_GSR_Histag_term COMPLETE

Tuesday, October 18, 2016 4:48 PM

Some of my members went to NYMU to work on cloning during July after our sponsors left Taiwan after June Work session. They cloned the whole construct and sent out for mutagenesis to Mission Bio tech. The DNA we received back from mutagenesis contained all the necessary parts. The construct for is

Bba_K880005_GSR_Histag_term COMPLETE!

Bba_K880005_CH25H_Histag_B0015

Tuesday, October 18, 2016 5:03 PM

CH25H (commercial) Transformation

Wednesday, March 23, 2016 3:29 PM

Goal: To transform the DNA received from Sino Biological Inc. into a competent cell

Procedure:

Centrifuge the DNA 5000x G for 5 min

Dissolve the DNA by putting in 100ul of water and pipetting

Incubate the DNA in room temperature for 10 min

Briefly Vortex then run a quick centrifuge under 5000 x G

Then, follow the same Cloning cycle procedure with the diluted DNA as the "Ligation Mix"

Result:

1 DNA mix
1 Transformation Control

Conclusion

Next step -> PCR Check and Gel Check

CH25H (commercial) PCR Phusion

Friday, May 20, 2016 2:27 PM

Goal: To elongate CH25HC to make more of them

Procedure: Thermo- PCR Phusion

Conclusion

Next Step -> PCR Clean up

CH25H (commercial) PCR Clean Up

Friday, May 20, 2016 2:30 PM

Goal: To PCR Clean up

Procedure:

Procedure

1. Transfer 100 µl (pcr mix) to 1.5 ml microcentrifuge tube
2. Add 5X volume (of pcr mix) Gel/PCR buffer
3. Vortex
4. If turned yellow add 10µl 3M Sodium Acetate (in the kit)
5. Transfer sample into 2ml collection tube and DFH column
6. Centrifuge 14-16,000 xg for 30sec
7. Discard flow through, place column back into centrifuge tube
8. Add 600µl wash buffer (w/ ethanol)
9. Let sit for 1 min
10. Centrifuge 14-16,000 xg for 30 sec
11. Discard flow through
12. Centrifuge again for 3 min
13. Transfer into 1.5ml centrifuge tube
14. Add 20-50µl elution buffer to center
15. Let sit for at least 2 min
16. Centrifuge 14-16,000 xg for 2 min
17. DONE!

Results

- [sample 1] = 11.4 ng/µl
- [sample 2] = 9.8 ng/µl

CH25H (commercial) Re-run PCR Phusion and PCR clean up redo 2

2016年5月27日　　上午 08:46

Goal: To run Phusion PCR with PCR Cleaned up DNA Segment as TEMPLATE DNA

Procedure: Thermo Fisher Scientific PCR Phusion

Result:

Segment of plasmid containing CH25H cDNA is amplified.

Conclusion:

Will be digested to put into igem biobrick backbone

CH25H (commercial) Phusion PCR and PCR Clean up redo

Thursday, May 26, 2016 3:39 PM

Goal: To add restriction sites to CH25H to clone it into igem biobrick backbone

Procedure:

Thermo Fisher Scientific Phusion PCR

Conclusion:

Relatively Low. The amplified DNA will be used as Template DNA for the next round of Phusion PCR.

CH25H (w/ Restriction sites) digestion/gel extraction/ligation/ transformation into iGEM bio brick backbone

Monday, June 06, 2016 1:13 PM

CH25H (in igem biobrick backbone) miniprepped for sequencing...

Wednesday, October 19, 2016 12:45 AM

CH25H Digestion w/ BBA_K880005 (vector) & Gel extraction

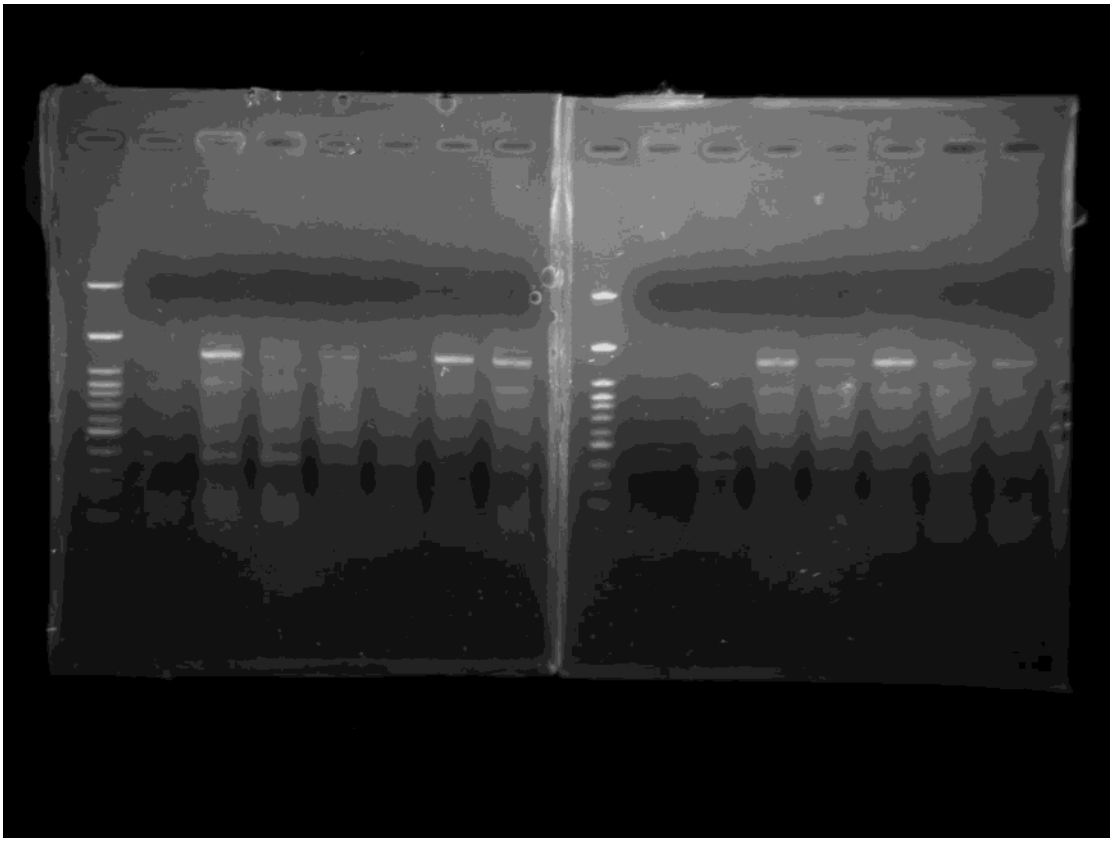
2016年6月16日　　下午 04:38

CH25H and Bba_K880005 Ligation and Transformation

2016年6月16日　　下午 04:39

Bba_CH25H PCR Gel Check

Monday, June 20, 2016 12:04 PM



All lanes contain Bba_CH25H PCR reaction mix. The desired band for CH25H is

Bba_K880005_CH25H Miniprep for sequencing

2016年6月17日　　上午 10:42

Bba_K880005_CH25H miniprep after 2 in 1, digestion, ligation, transformation into 10

2016年6月15日　　上午 11:59

Concentrations of miniprep:

- Bba_ch25h 8: 114.6 ng/ml
- Bba_ch25h 10: 151.5 ng/ml

Digestion:

Miniprep concentration (ng/uL)	**DNA (uL)**	**Water (uL)**
114.6	8.73	7.27
151.5	6.60	9.40

Ligation:

	Lig (uL)	**LC (uL)**
Buffer	2	2
Enzyme	1	1
Vector	3.7	3.7
Insert	5	0
Water	9.3	14.3

TRANSFORMATION WORKED!

Cloning Cycle Redo

Wednesday, June 29, 2016 12:19 PM

All the previous cloning cycle including Bba_K880005 and His Tag Term were all shut down because the sequencing revealed that we did not have both part.

The individual part, CH25H in iGEM Backbone/Vector was correct.

Bba_K880005_CH25H_Histag_term

Tuesday, October 18, 2016 4:53 PM

IDT offered free manufacturing of composite parts to iGEM teams. We used this opportunity to ask IDT to construct Bba_K880005_CH25H_Histag_B0015. Without the need of our own cloning, we received our full construct.

Bba_K880005_CH25H_Histag_term completed!

Bba_K880005_CRYAB_Histag_term

Tuesday, October 18, 2016 5:31 PM

5/19 CRYAB Transformation

Tuesday, October 18, 2016 7:53 PM

PCR Phusion and PCR Clean Up for CRYAB (commercial)

Friday, May 20, 2016 2:01 PM

Goal: To add EXSP Restriction Sites to Commercial CRYAB

Procedure:

Based on Thermo - Phusion PCR 2 step Protocol with 20ul Rxn mix

Goal: To PCR Clean up CRYAB to get a segment of Plasmid DNA that contains DNA to express CRYAB

Procedure:

Procedure

1. Transfer 100 µl (pcr mix) to 1.5 ml microcentrifuge tube
2. Add 5X volume (of pcr mix) Gel/PCR buffer
3. Vortex
4. If turned yellow add 10µl 3M Sodium Acetate (in the kit)
5. Transfer sample into 2ml collection tube and DFH column
6. Centrifuge 14-16,000 xg for 30sec
7. Discard flow through, place column back into centrifuge tube
8. Add 600µl wash buffer (w/ ethanol)
9. Let sit for 1 min
10. Centrifuge 14-16,000 xg for 30 sec
11. Discard flow through
12. Centrifuge again for 3 min
13. Transfer into 1.5ml centrifuge tube
14. Add 20-50µl elution buffer to center
15. Let sit for at least 2 min
16. Centrifuge 14-16,000 xg for 2 min
17. DONE!

Results

- [sample 1] = 15.5 ng/µl

Conclusion

Run Digestion and put the part into Bba_K880005 iGEM Backbone to make BBa_CRYAB construct

Cloning Cycle Redo

Wednesday, June 29, 2016 12:19 PM

All the previous cloning cycle including Bba_K880005 and His Tag Term were all shut down because the sequencing revealed that we did not have both parts.

GSR in iGEM Backbone/Vector was correct.

Bba_K880005_CRYAB_Histag_term

Tuesday, October 18, 2016 6:42 PM

Some of my members went to NYMU to work on cloning during July after our sponsors left Taiwan after June Work session. They cloned the whole construct and sent out for mutagenesis to Mission Bio tech. The DNA we received back from mutagenesis contained all the necessary parts. The construct for

Bba_K880005_CRYAB_Histag_term is COMPLETE!

Bba_K880005_GFP_Histag_Term

Wednesday, October 19, 2016 12:15 AM

Goal: To make a full iGem bio brick of BBa_k880005 , GFP Gene , and 10x HIs-tag to test whether the 10x His-Tag protein purification protocol works. GFP is fluorescent so it is easy to tell whether the purification works even without carrying out protein analysis like for other transparent proteins.

10xHis-tag and GFP gene (vector) digestion

Monday, March 7, 2016 3:17 PM

Goal: To digest 10x His-tag

Same Protocol As Cloning Cycle

Sizes

- GFP: 750bP
- His-tag_TT : 36bP

Volume (uL)

	DNA	Water
GFP	5.32	10.68
His-tag	5.94	10.00

Cutting sites:

- GFP: S&P
- His-tag: X&P

Designed a Protocol for use, Check on Wood Cabinet for exact procedure. (There will be an extra step that includes terminator as the fourth one.)

10xHis-tag (backbone) and GFP gene (insert) digestion Re-do

Wednesday, March 9, 2016 5:24 PM

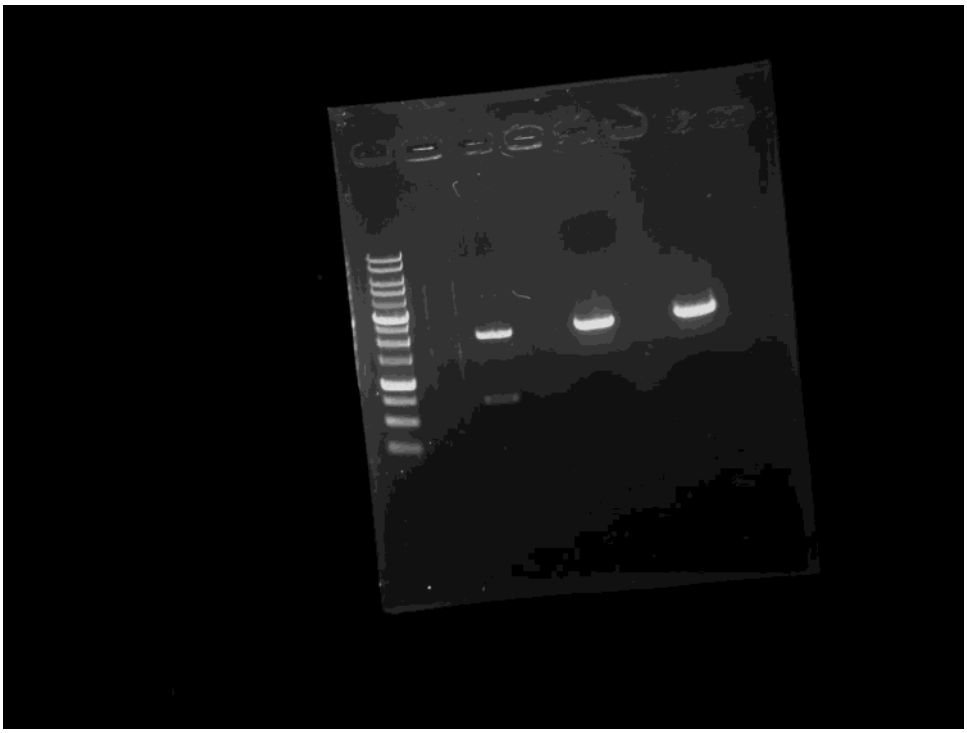
Goal: To design a procedure of creating the Construct and hopefully start digestion

Sizes:
GFP: 720 bp
His Tag + Stop Codon: 40 bp
Promoter+RBS: 55 bp
Terminator + Backbone: ~´2100 bp

10xHis-tag and GFP gel purification

Tuesday, March 08, 2016 10:56 AM

Goal: To run gel for digestion mix to cut out the vector (10x His-Tag + Backbone) + insert (GFP gene)



Lane	1	2	3	4	5	6	7	8
Substance	1kb Ladder		GFP		10xHIS		10xHIS	
Band Size	N/A		720		~2106bp		~2106bp	

Procedure:

Cut out GFP (E&S) and 10xHIS (EX)

Stored in 4c in the lab in the box called "igem Exp. In progress"

10x His-tag and GFP Gel Extraction

2016年3月10日　　下午 02:44

Goal:

To extract and purify the 10x His Tag gels and GFP gels, and potentially start ligation process

10x His-tag and GFP Ligation + Transformation

2016年3月11日　　下午 03:30

Goal: To ligate the 10x His tag vector and GFP insert to make a part which will be ligated into BBa_K880005 backbone (vector)

Procedure:

Ligation

Same as that of Cloning Cycle

Transformation (old cells protocol)

Same as that of Cloning Cycle

Result

Concentration of the digested vector and insert

10xHIS (Vector)	11.6 ng/uL	~2100 bps
GFP (Insert)	3.1 ng/ul	720 bps

Components for ligation mix

(uL)	**Ligation Mixture**	**Negative Control**
Buffer	2	2
Enzyme	1	1
Water	4.4	14.4
10xHIS	2.6	2.6
GFP	10	0

Conclusion

Next step = Storage in Incubator 37c overnight, Taken out the next day, Stored in 4c fridge

Next step = PCR check -> Gel Check -> Miniprep -> (Go through the cloning cycle process with BBa_K880005 backbone (vector))

GFP_10x His-Tag Gel Check & Miniprep

Thursday, March 17, 2016 3:29 PM

Goal : To check whether the 10x His- Tag (vector) and GFP (Insert) are combined

Procedure:

Voltage: 130 V

Time: 15min

Result:

Conclusion:

Next Step -> Redo PCR Check just to make sure

Goal: To make liquid culture for miniprep to start the cloning cycle for GFP + 10x His-Tag with BBa_K880005 (already gel purified DNA)

Procedure:

Liquid culture for cloning cycle

Stored in shaker 37C over night

Conclusion

Next Step -> Miniprep

GFP_10x His-Tag Miniprep

Thursday, March 17, 2016 3:33 PM

Goal: To prepare the plasmid DNA sample of the full construct of GFP + 10x His-TAG for later CLONING CYCLE WITH BBa_K880005

Procedure:

Same as Cloning Cycle

Result:

1 sample of Miniprep

Concentration: 211.7 ng/ul

Conclusion:

Next Step -> Digestion

GFP_10x His tag Digestion, Gel Extraction

Thursday, March 17, 2016 3:34 PM

Goal: To cut out the GFP + 10x His- Tag as inserts for the construct BBa_K880005 + GFP + 10x His- Tag

Procedure:

Digestion: Same as Cloning cycle

- Amount of DNA: 1000ng / (211.7ng/ul)= 4.72ul

Gel Extraction: Geneaide Gel Extraction Kit Protocol (From now will be used for Cloning Cycle)

Histag and bba liquid culture

Result:

Gel Run Photo + Label to be posted...

Conclusion:

Next step -> Ligation + Transformation with BBa_K880005

GFP_10x His-Tag Gel Run and cut

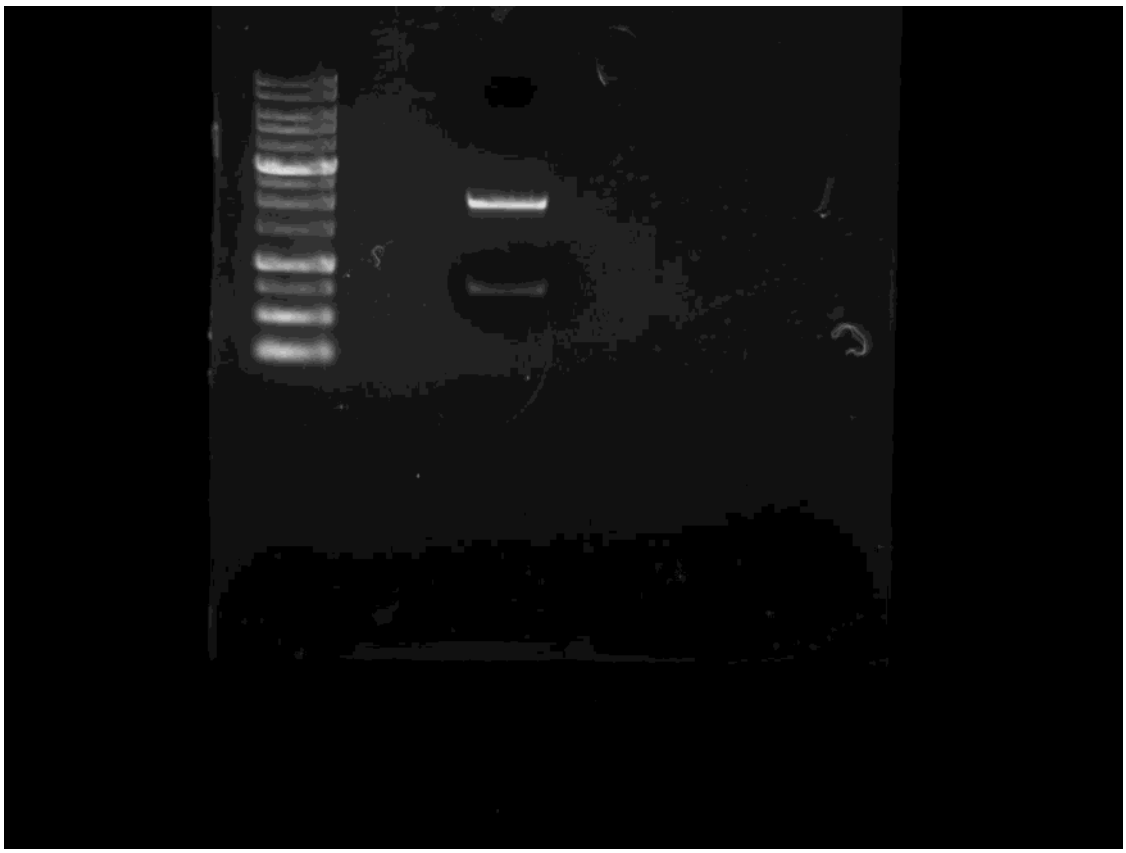
Tuesday, March 22, 2016 12:44 PM

Goal: To cut out the insert from the GFP + His-Tag part to put into the Bba_K880005 backbone using ligation and transformation

Procedure:

Same as Cloning Cycle

Result:



Lane	1	2	3
Substance	1kb Ladder		iGEM Bio Brick Backbone
			GFP + 10x His-tag
Band Size	N/A		~2120bp
			~760bp

Conclusion

Next Step -> Gel purification of the insert

Gel Purification & Ligation of GFP/10xHisTag BBaK880005

Tuesday, March 22, 2016 2:52 PM

Goal:
Ligate Vector and Insert

Procedure:

Two tubes Ligation Mixture and Ligation Ctrl

Ligation Calculator

Total Reaction Mix(µl)	20
Volume of the buffer(µl)	2
Volume of the enzyme(µl)	1

	Vector	Insert
Molar Ratio	1	3
Concentration(ng/µl)	16.2	2.5
Length(bp)	2155	800
Maximum volume(µl)	5	10

Known Vector Lengths: pET-32 | pET-28 | pMAL-c2

Ligation Mix

	Vol. in µl
Buffer	2
Enzyme	1
Vector	1.4
Insert	10
Water	5.6
Total	20

Insert is GFP/10x His Tag which is 2.5 ng/ul
Vector is BBaK880005 which is 16.2 ng/ul

Result:
After ~1 hours, put into freezer

Next:
Transformation tomorrow

GFP_10x Histag Transformation with BBa_K880005

Wednesday, March 23, 2016 3:26 PM

Goal: To transform the ligated BBa_K880005 and GFP + 10x His-tag

Procedure:

Same as Cloning cycle procedure

Result:

1 Ligation Mix
1 Vector Control
1 Transformation Control

Conclusion:

Next Step -> PCR check, gel check -> Another cloning cycle to add the terminator

BBa_K880005_GFP_10x His-tag Miniprep and PCR/Gel Check

Monday, April 04, 2016 11:30 PM

Goal: To Miniprep to obtain pure plasmid (to later transform it with a Terminator)

Procedure:

Same as ever

Result: (need to be Nano dropped)…

Conclusion:

Goal: To check whether Bba_GFP_10x His was transformed correctly

Procedure:

Colony PCR (same as ever) - BUT adjust the length of the Extension time according to the length of the part

Result:

Conclusion:

Proves that Pure Plasmid is all right -> Can carry on with further experiments with the PP already miniprepped

BBa_K88005_GFP_10x His tag Concentration and Digestion + Digestion of B0015

Wednesday, April 6, 2016 3:22 PM

Concentration of the BBa_GFP_10x His tag :

PP 1: 371 ng/uL
PP 2: 429.3 ng /uL
PP 3: 410.8 ng/ uL

Goal: Digestion to put insert, BBa_K88005_GFP_10x His tag, into the vector (terminator) B0015

Procedure:
Same as ever

Result:

Insert
We used pp2 because it was the highest concentration.

DNA	2.33
Restrictor E	1.0
Restrictor S	1.0
10x NE Buffer	2.0
Distilled Water	13.07

Vector

DNA	4.92
Restrictor E	1.0
Restrictor X	1.0
10x NE Buffer	2.0
Distilled Water	11.08

Conclusion:

Next Step -> Run Gel for Gel Extraction

Bba_K880005_GFP_10x His tag & B0015 Gel Run

Friday, April 08, 2016 3:13 PM

Goal : To check whether the Bba_K880005_GFP_10x His tag was cut out properly

Procedure:

Same Gel run procedure as the Cloning Cycle

Result:

Only ran the insert Bba_GFP_10x His tag & B0015

Conclusion

- The cut out insert did not show its approx. 1kb band
- Only the backbone could be seen
- Have to Re-digest Bba_GFP_10x His tag

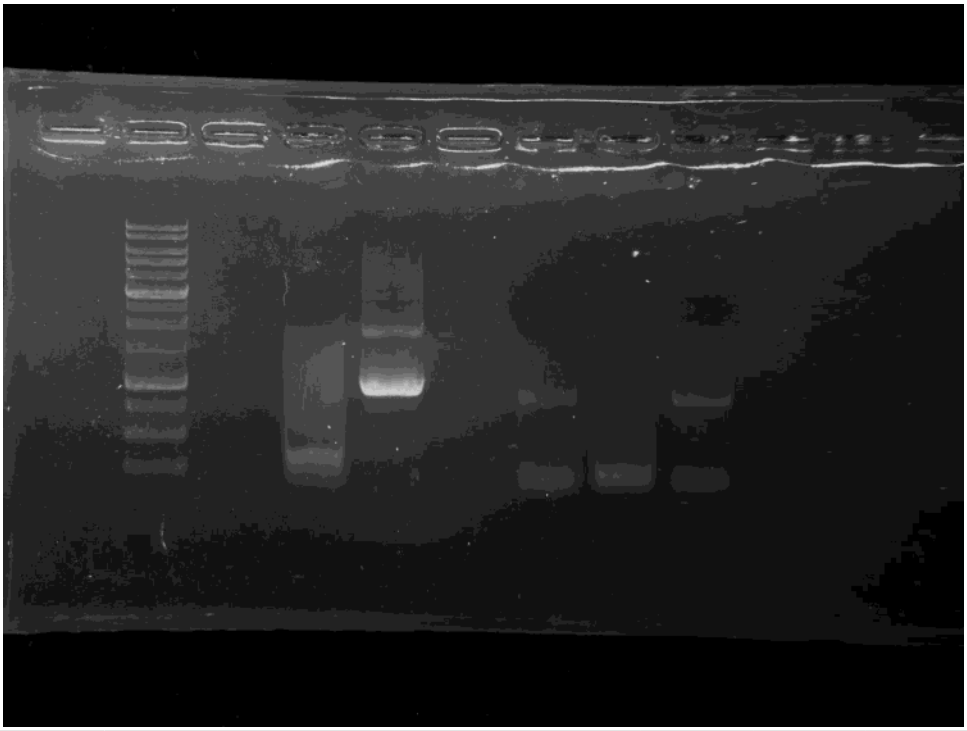
Next time -> Re Digest Bba… and Gel Run B0015 Vector

Bba_K880005_GFP_10x His tag & B0015 Gel Extraction

Wednesday, April 20, 2016 2:00 PM

BBaK880005_GFP_His_B0015 PCR gel check

Thursday, April 21, 2016 3:38 PM



Ladder		Vector	GFP(positive		3	4	5(expected

The band is shorter than expected. We have sent it out for sequencing.

IDT GFP commercial full construct (Bba_K880005 _GFP_Histag_Terminator) Transformation into iGEM Backbone

2016年9月8日　　下午 04:47

IDT offered free manufacturing of composite parts to iGEM teams. We used this opportunity to ask IDT to construct Bba_K880005_GFP_Histag_B0015. Without the need of our own cloning, we received our full construct.

Experiment Log on the full construct

9/6 Transformed the construct into Elite competent cell
9/7 Liquid Cultured the cells.
9/8 Plasmid Purified the liquid culture

Bba_K880005_CRYAA_His-tag_B0015

Sunday, October 16, 2016 1:34 PM

Origene CRYAA cDNA Basic Information

Sunday, February 28, 2016 3:00 PM

Origene CRYAA was obtained in a vile in 10mg powder form.

ORF Size	522bp
Antibiotic Resistant	Kanamycin
Primers	Designed
Reference Sequence	NM_000394
Storage	Lab -20C Fridge (Counteracts Box) along with its primers

Further Information

http://www.origene.com/human_cdna/NM_000394/SC300064/CRYAA.aspx

Origene CRYAA cDNA Transformation

Wednesday, February 24, 2016 2:53 PM

Goal

- To insert Origene CRYAA cDNA into bacterial cells so that the cDNA can be infinitely reproduced.

Procedure

- 10mg of CRYAA cDNA reconstituted with 100ul water: 100ng/ul
- Transformation (new protocol: non-heat shock)
  - Thaw competent cell vial (100ul total) --- transfer 50ul to clean new tube (**Fire On**)
  - Add DNA (less than 10% of volume of cells). Vortex 1 second (**Fire On**)
    - Add 1ul DNA (@100 ng/ul)
  - Place on ice for 10min
  - Plate all transformation mix on LB+ Kanamycin using glass beads (**Fire On**)
  - Incubate overnight at 37 degrees Celsius

Conclusion

Pending for PCR Check to determine whether CRYAA is expressed in E.coli

Origene CRYAA cDNA PCR Check

Friday, February 26, 2016 3:09 PM

Goal

- To certify that the cDNA is properly expressed in the cell.

Procedure

- 2x Taq Polymerase PCR
- Before putting in 1 bacterial colony, the colony was streaked on another plate to continue the cultivation of the Origene CRYAA cDNA bacteria

IMPORTANT:

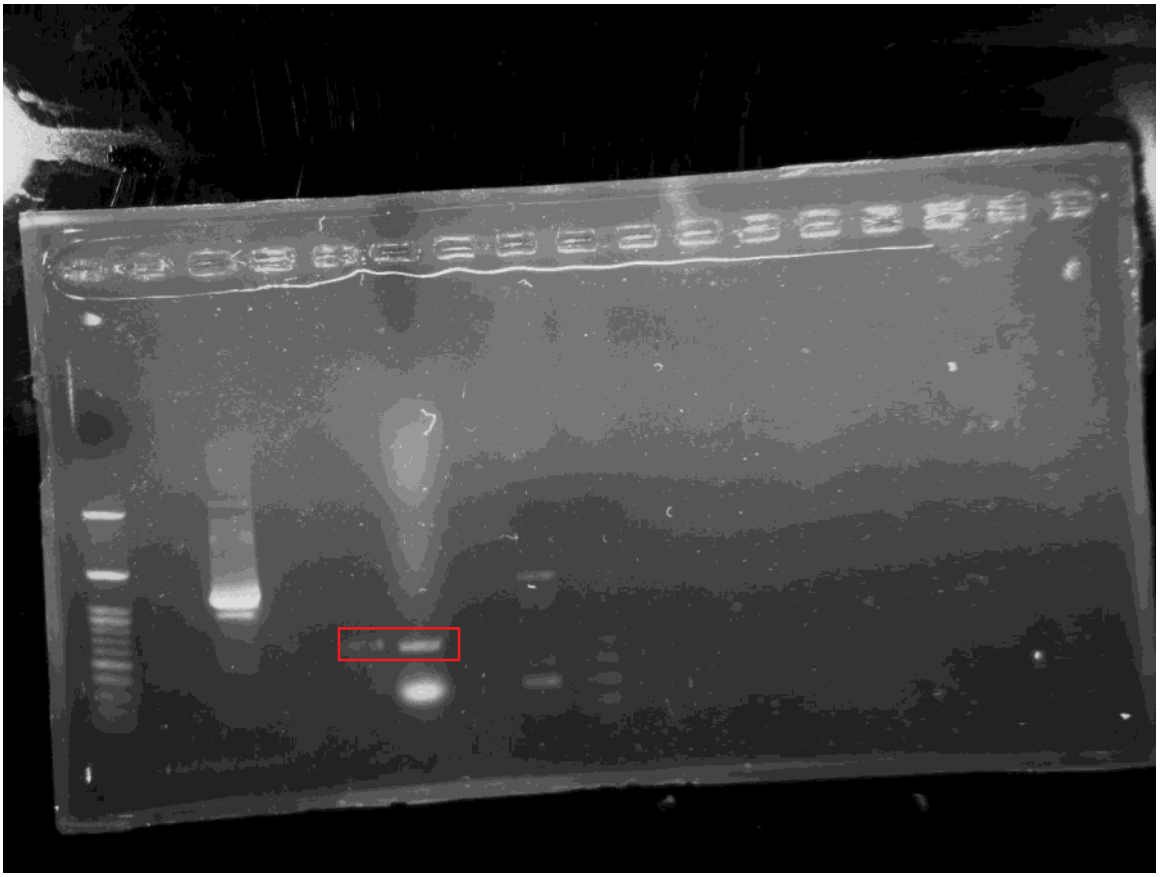
For the Bba_880005 PCR Check it was carried out with CRYAA and GFP Gene (**Control)**

- The time of the **Extension (step 4**) should be modified according to the longest DNA that must be elongated
  -
  - The standard for modification is 1 min to make 1 kb DNA
- In this case, the longest DNA to be elongated is that of GFP Gene: approximately 1kb
  - The time for extension is **1 min**

**The length of the DNA is found through Ordering Info and iGEM Parts Website*

Origene CRYAA cDNA Gel Check & Liquid Culture

Monday, February 29, 2016 8:14 PM



Lane	1	2	3	4	5	6	7	8	9
Substance	100bp DNA Ladder		GFP Gene (Control)		CRYAA (Restreak 1)	CRYAA(Restreak 2)		Bba_K880005 (Restreak 1)	Bba_K880005 (Restreak 2)
Band Size	N/A		~1kbp		~522bp	~522bp		~355bp	~355bp

The bands around 500bp were seen in Lane 5 and Lane 6. The CRYAA were correctly transformed.

Origene CRYAA Miniprep

Monday, February 29, 2016 8:36 PM

Goal: To obtain Pure plasmid of Origene CRYAA for Phusion PCR to add Restriction sites

Procedure:

Geneaid Miniprep Kit protocol

Conclusion:

Concentration

RESTREAK 1 CRYAA 1: 740.3 ng/ul

RESTREAK 1 CRYAA 2: 487.7 ng/ul

RESTREAK 2 CRYAA 1: 985.9 ng/ul

RESTREAK 2 CRYAA 2: 552.6 ng/ul

CRYAA PCR (Phusion DNA Kit)

Wednesday, March 09, 2016 1:25 PM

Goal: To carry out PCR with CRYAA to create CRYAA cDNA with restriction sites so that it can be put into iGem Bio Brick backbone (which requires E,X,S,P restriction sites)

20ul Sample

PCR Mix Component (Refer to Phusion High-Fidelity DNA polymerase Protocol)

List	Amount (20ul)	Amount (50ul)
H2O	DNA + H20 = 14.4 ul	
5x Phusion HF Buffer	4ul	
10uM dNTPs	0.4ul	
Forward Primer (100mM) EX CRYAA	0.5ul	
Reverse Primer (100mM) SP CRYAA	0.5ul	
Template DNA	100ng needed	
Phusion DNA Polymerase	0.2ul	

Template DNA concentration

Colony	Amount (ul)
CRYAA 1	100ng/ (740.3ng/ul) = 0.14
CRYAA 2	0.21
CRYAA 3	0.10
CRYAA 4	0.19

PCR Cycle Steps (We follow 3 step protocol and the ones highlighted)

	Cycle Step	2 step		3 step protocol		Cycle

		protocol				
		Temp. C	Time	Temp	Time	-
1	Initial Denaturation	98	30 sec	98	30sec	1
2c	Denaturation	98	5-10sec	98	5-10sec	
3c	Annealing	-	-	74 (Differs for every DNA - Usually Lower melting temperature of the primers +3) This temp. Must be between 55-68c	10-30sec	25-35 30
4c	Extension	72	15-30sec/kb	72	15-30s/kb (Product will be around 800bp)	
5	Final Extension	72	5-10min	72	5-10min	1
6	Storage	4	Hold (press clear on the machine)	4	Hold (press clear on the machine)	-

CRYAA PCR Clean Up

Tuesday, March 08, 2016 10:55 AM

Purpose

- Purify CRYAA DNA

Procedure

1. Transfer 100 µl (pcr mix) to 1.5 ml microcentrifuge tube
2. Add 5X volume (of pcr mix) Gel/PCR buffer
3. Vortex
4. If turned yellow add 10µl 3M Sodium Acetate (in the kit)
5. Transfer sample into 2ml collection tube and DFH column
6. Centrifuge 14-16,000 xg for 30sec
7. Discard flow through, place column back into centrifuge tube
8. Add 600µl wash buffer (w/ ethanol)
9. Let sit for 1 min
10. Centrifuge 14-16,000 xg for 30 sec
11. Discard flow through
12. Centrifuge again for 3 min
13. Transfer into 1.5ml centrifuge tube
14. Add 20-50µl elution buffer to center
15. Let sit for at least 2 min
16. Centrifuge 14-16,000 xg for 2 min
17. DONE!

Results

- [sample 1] = 11.4 ng/µl
- [sample 2] = 9.8 ng/µl

Conclusion

- Concentrations too low to run digestion, even if we combine the two samples
- We need to run another PCR for higher concentrations to continue with digestion

CRYAA PCR Re-Run (w/ Control)

Wednesday, March 9, 2016 4:42 PM

Goal:
To carry out PCR with CRYAA to create CRYAA cDNA with restriction sites so that it can be put into iGem Bio Brick backbone (which requires E,X,S,P restriction sites)

One 20ul Sample
Only worked with one sample with the lowest concentration since once PCR starts remaining DNA is required and hence to not waste as much DNA.

PCR Mix Component (Refer to Phusion High-Fidelity DNA polymerase Protocol)

List	CRYAA Amount (20ul)	List	GFP Gene Amount (50ul)
H2O	DNA + H20 = 14.4 ul	H2O	DNA + H20 = 14.4 ul
5x Phusion HF Buffer	4ul	5x Phusion HF Buffer	4ul
10uM dNTPs	0.4ul	10uM dNTPs	0.4ul
Forward Primer (10mM) **Diluted from 100uM** EX CRYAA	1ul	Forward Primer (10mM) **Diluted from 100uM** VF2	1ul
Reverse Primer (10mM) **Diluted from 100uM** SP CRYAA	1ul	Reverse Primer (10mM) **Diluted from 100uM** VR	1ul
Template DNA	100ng needed	Template DNA	100ng needed
Phusion DNA Polymerase	0.5ul	Phusion DNA Polymerase	0.5ul

Template DNA concentration

Colony	Amount (ul)
CRYAA 2	100ng/(487.7ng/ul)=0.21

PCR Cycle Steps (We follow 3 step protocol and the ones highlighted)

	Cycle Step	2 step protocol		3 step protocol		Cycle
		Temp. C	Time	Temp	Time	-
1	Initial Denaturation	98	30 sec	98	30sec	1
2c	Denaturation	98	5-10sec	98	5-10sec	
3c	Annealing	-	-	60 (**Important**: This temperature	10-30sec	25-35

				is the melting temperature +~ 3c of only the anneling codons of the primer with the lower melting temperature (F primer)) Refer online protocol for further (Differs for every DNA - Usually Lower melting temperature of the primers + 3) This temp. Must be		30
4c	Extensio n	72	15-30sec/k	72	15-30s/kb (Product will be around 800bp)	
5	Final Extensio	72	5-10min	72	5-10min	1
6	Storage	4	Hold (press clear on the machine)	4	Hold (press clear on the machine)	-

CRYAA PCR Clean Up & Digestion with BBa_K880005 Backbone as a vector

Tuesday, March 08, 2016 10:55 AM

Purpose

- Purify PCR DNA

Procedure

1. Transfer 100 µl (pcr mix) to 1.5 ml microcentrifuge tube
2. Add 5X volume (of pcr mix) Gel/PCR buffer
3. Vortex
4. If turned yellow add 10µl 3M Sodium Acetate (in the kit)
5. Transfer sample into 2ml collection tube and DFH column
6. Centrifuge 14-16,000 xg for 30sec
7. Discard flow through, place column back into centrifuge tube
8. Add 600µl wash buffer (w/ ethanol)
9. Let sit for 1 min
10. Centrifuge 14-16,000 xg for 30 sec
11. Discard flow through
12. Centrifuge again for 3 min
13. Transfer into 1.5ml centrifuge tube
14. Add 20-50µl elution buffer to center
15. Let sit for at least 2 min
16. Centrifuge 14-16,000 xg for 2 min
17. DONE!

Results

- Concentration = 44.4 ng/ul

Conclusion

- Next step = digestion

Purpose

- To digest CRYAA PCR Clean up sample and BBa_k880005 for a vector

Procedure

1. Make a separate digestion mix (same as cloning cycle digestion) for CRYAA PCR and BBa_k880005 miniprep sample both with restriction sites as E, and P
2. Place both digestion mix in a 37c incubator for at least an hour
3. Store the mixes in 4c overnight

Results

- Concentration of CRYAA PCR Clean up : 1000ng/ (44.4ng/ul) = 22.52 ul
- Concentration of BBa_k880005 : 1000ng/ (441.7ng/ul) = 2.26 ul

Conclusion

- Next step = Gel run to cut out the insert (CRYAA DNA) and the vector (BBa_k880005)

CRYAA DNA (insert) Gel Run and Purification

Friday, March 11, 2016 5:01 PM

Goal

- **To separate out the cut vector BB_k88005 from the gel and purify it**

Procedure

Ran gel for BBa_K880005 Backbone (Vector)
Write down Voltage, and time taken to run the gel

Write down the protocol for gel extraction or just say "same as that of cloning cycle"

Result

Conclusion

Stored in 4c Igem exp. In progress box

Gel Purification: 880005, CRYAA (x2)
Concentrations:

880005	CRYAA	CRYAA
12.0	8.4	8.3

CRYAA and Bba_K880005 Ligation and Transformation

Thursday, March 17, 2016 2:41 PM

Goal: To ligate CRYAA (insert) into BBa_K880005 (vector) and complete iGEM Bio Brick Part for CRYAA

Procedure: Same as Old Cloning Cycle "Transformation after Ligation"

Result:

Concentration of the digested vector and insert

BBa_K880005 (Vector)	12.0 ng/uL	~2120 bps
CRYAA (Insert)	8.4 ng/ul	522bps

Components for ligation mix

(uL)	**Ligation Mixture**	**Negative Control**
Buffer	2	2
Enzyme	1	1
Water	4.4	4.4
BBa_K880005	2.6	2.6
CRYAA	10	0
H20	0	10

Picture to be posted

1. Ligation
2. Vector Control
3. Transformation Control

Conclusion:

The transformation was successful

Next step -> PCR Check (Taq) and Gel Check

CRYAA (in iGEM backbone) PCR Check

Thursday, March 17, 2016 3:22 PM

Goal: To check whether CRYAA is expressed in the BBa_k880005

Procedure: Same as Cloning Cycle Colony PCR Check

Conclusion:

Gel Check for the PCR Check

CRYAA (in IGEM backbone) Liquid Culture

Tuesday, March 22, 2016 12:40 PM

Goal: To make Liquid culture to make pure plasmid using Miniprep Process

Procedure:

Same as Cloning Cycle Liquid Culture

Result:

Two liquid cultures of CRYAA (from same colony)

Conclusion

Next Step -> Miniprep

CRYAA (from now on in iGEM backbone) Miniprep/Restock

Tuesday, March 22, 2016 12:42 PM

Goal: Miniprep to make pure plasmids of CRYAA (now it is under Chloramphenicol since it is in iGEM bio brick backbone)

Procedure:

Same as Cloning Cycle Miniprep

Result:

Concentration

CRYAA 1 208.8 ng/ul

CRYAA 1 200.8 ng/ul

Conclusion

Next step -> NEED TO redo for sequencing

CRYAA Liquid Culture for Sequencing

Wednesday, March 23, 2016 3:24 PM

Goal: To make liquid culture for CRYAA sequencing

Procedure: Same as Cloning cycle

Result:

1 tube of liquid culture

Conclusion

Next step -> Sent out for sequencing

CRYAA MiniPrep for Sequencing

Thursday, March 24, 2016 3:15 PM

Goal:

- Miniprep CRYAA for sequencing

Procedure:

- Same as normal Miniprep but use distilled H2O instead of Elution Buffer

Result:

- Successfully Miniprepped

Concentrations: 127.3 ng/ul

Placement: The orange box

CRYAA and B0015 Digestion

Thursday, March 24, 2016 3:31 PM

Goal

- To digest CRYAA (with restrictions enzymes E and S) and terminator B0015 (with restriction enzymes E and X). This will be followed by ligation to combine the promoter, RBS, CRYAA, and B0015 terminator on the BBa_K880005 vector backbone

Procedure

- Same as ever

Results

- Success
- [B0015] = 24.1 ng/ul
- [P + R CRYAA] = 40 ng/ul

Conclusion

- Next step is ligation

CRYAA and B0015 Ligation and Transformation

Monday, April 04, 2016 11:22 PM

Goal: To put a terminator behind CRYAA to regulate the production of CRYAA

Procedure:

Same as ever (Cloning Cycle)

Result:

Ligation Mixture Component

	Ligation (ul)	Vector (ul)
Buffer	1	1
Vector	4	4
Insert	4	N/A
ddh20	N/A	4
Ligase	1	1
Total	10	10

Conclusion:

Next Step -> Colony PCR Check

CRYAA_B0015 PCR Check

Thursday, April 14, 2016 5:58 PM

Goal: To check whether the terminator was added to CRYAA

Bba_K880005 and CRYAA_B0015 Ligation + Transformation

Wednesday, April 20, 2016 1:59 PM

Insert - CRYAA
Vector - BBa

Ligation Calculator

Total Reaction Mix(µl)	20
Volume of the buffer(µl)	1
Volume of the enzyme(µl)	1

	Vector	Insert
Molar Ratio	1	3
Concentration(ng/µl)	10.7	1.0
Length(bp)	2120	800
Maximum volume(µl)	10	10

Known Vector Lengths: pET-32 | pET-28 | pMAL-c2

Ligation Mix

	Vol. in µl
Buffer	1
Enzyme	1
Vector	0.8
Insert	10
Water	7.2
Total	20

Bba_K880005_CRYAA Digestion

Monday, May 2, 2016 3:10 PM

To check transformation of BBa + CRYAA
PCR

Control	GFP term
Colony 1	
Colony 2	

Digestion

CMR	Sites: S,P	DNA: 1.62 (microliters)
GFP	Sites: X,S	DNA: 5.83

Bba_K880005_CRYAA Sequencing...

Wednesday, May 11, 2016 9:39 PM

Sequencing result showed that the cloning was not successful. And also, we forgot to clone His tag_Term behind CRYAA. More importantly there was stop codon behind CRYAA which meant that even after Histag is added, histidine tag will not be transcribed. We had to order reverse primers to get rid of that stop codon.

CRYAA (commercial) Reverse Primers ordered

Friday, May 20, 2016 2:05 PM

Goal: To purchase a reverse primer which does not replicate "Stop codon" and hence eliminate the stop codon from CRYAA

CRYAA Phusion PCR

Thursday, May 26, 2016 3:31 PM

Goal: To eliminate the stop codon from CRYAA and elongate CRYAA

Procedure: Thermo Scientific Phusion PCR

Phusion PCR CRYAA Re-do and PCR Clean up

Thursday, May 26, 2016 3:36 PM

Wrong Primer Concentration was used so Phusion PCR was redone

PCR Clean Up & Digestion w/ Bba_K880005 (vector)

Thursday, May 26, 2016 3:36 PM

Goal: To digest CRYAA DNA to later insert it behind Bba_K880005 in iGEM Backbone

Procedure:
Same as "Cloning Cycle" Digestion

Result:

Bba_K880005
Miniprep Concentration - 443.1 ng/ul
CRYAA
PCR Clean Up Concentration – 67.0 ng/ul

Conclusion:

Next step -> Gel Extraction

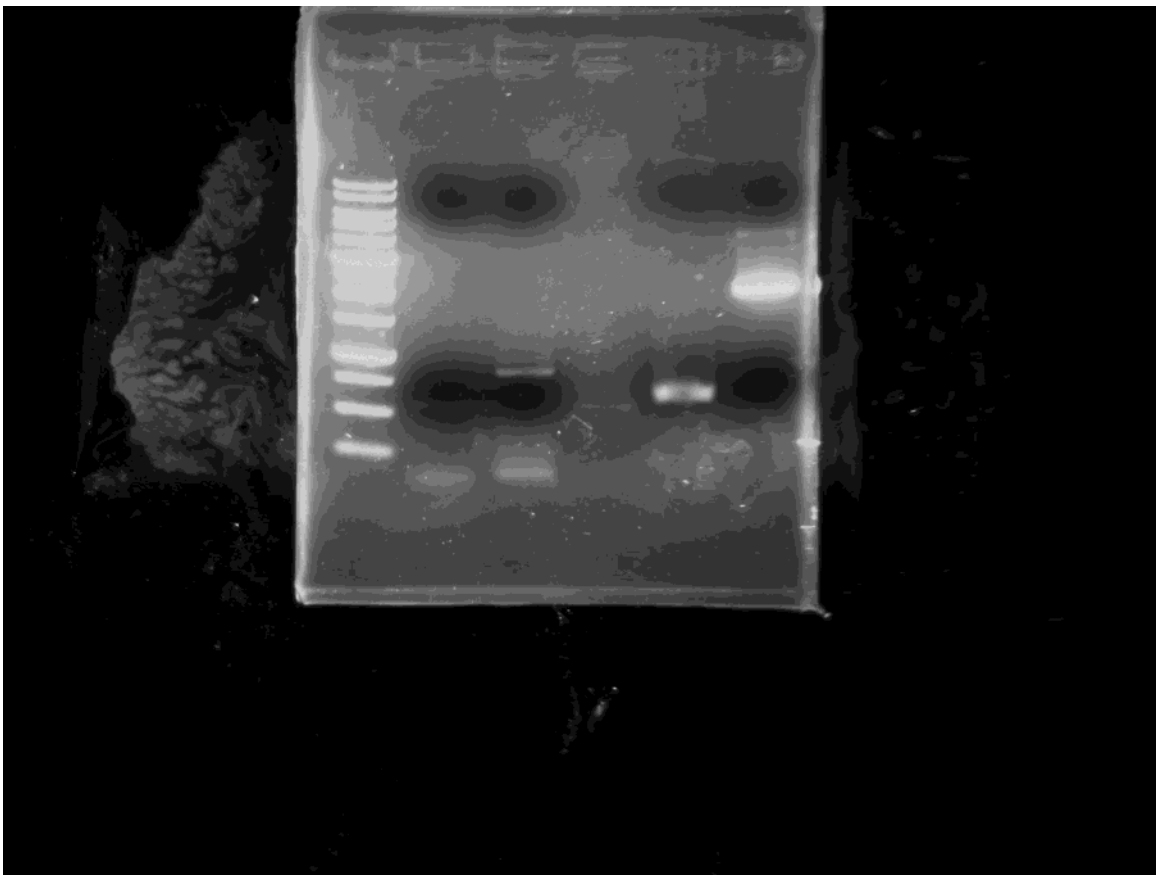
CRYAA and Bba_K880005 (vector) digestion

Monday, June 06, 2016 11:21 AM

Goal: To cut out the bands containing CRYAA DNA and Bba_K880005 IGEM Vector

Procedure:
Same as "Cloning Cycle"

Result:



Ladder	CRYAB	CH25H	N/A	CRYAA	Bba_K880005
1kb ladder	~500bp	~800bp	N/A	~800kb	~2100kb

- CRYAB and CH25H (positive controls) went through Taq Polymerase PCR check to see whether Phusion Primers worked in the first place
- All bands are correct.

Conclusion:

Next Step -> Gel Extract CRYAA and Bba_K880005 iGEM Backbone

CRYAA and Bba_K880005 (vector) Gel Extraction

Monday, June 06, 2016 11:22 AM

CRYAA and Bba_K880005 (vector) Ligation and Transformation

Monday, June 06, 2016 11:22 AM

PCR and Gel Check showed that

Re-digest CRYAA and Bba_K880005 & Gel extract

Monday, June 06, 2016 1:22 PM

Goal: To digest CRYAA DNA to later insert it behind Bba_K880005 in iGEM Backbone

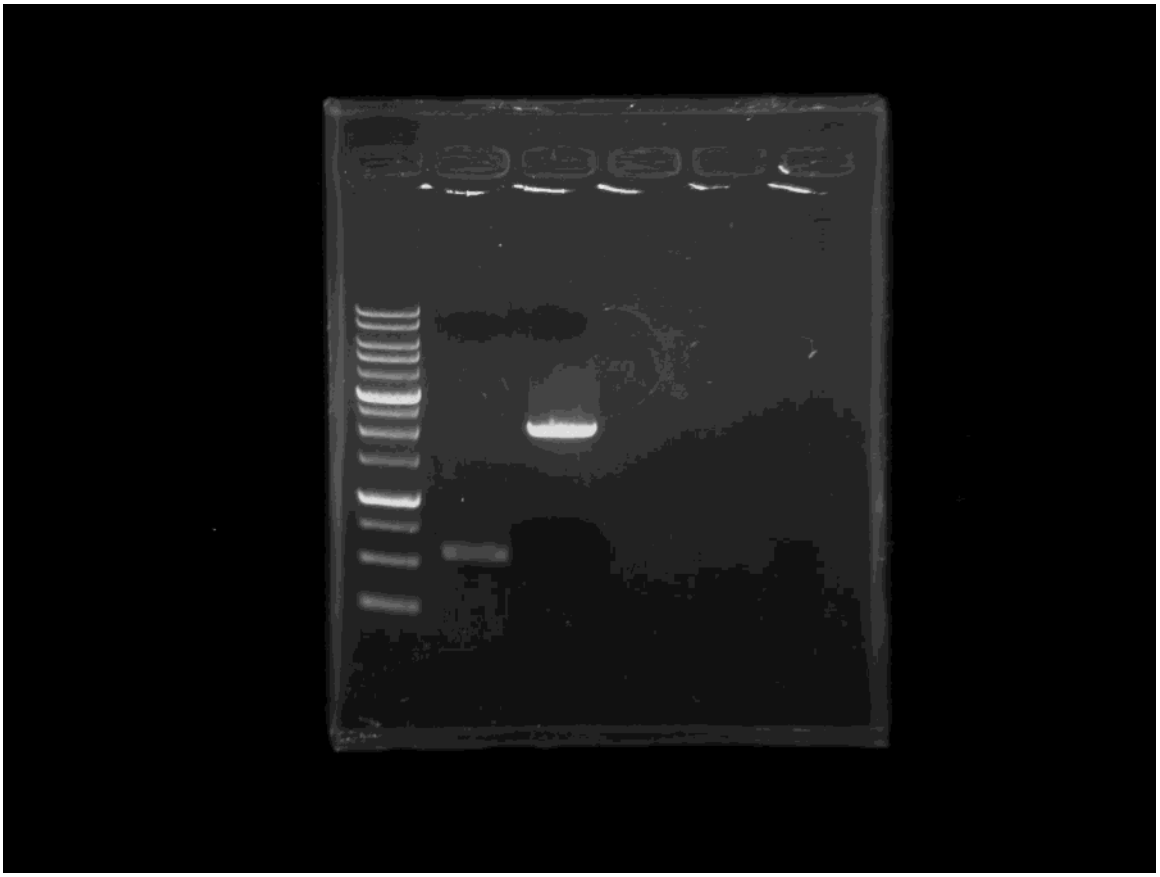
Procedure:
Same as "Cloning Cycle" Digestion

Result:

Digestion

Bba_K880005
Miniprep Concentration - 443.1 ng/ul
CRYAA
PCR Clean Up Concentration – 67.0 ng/ul

Gel Run Picture:



Lane 1: DNA Ladder
Lane 2: CRYAA (PCR Clean Up DNA Segment)
Lane 3: Bba_K880005 (Pure Plasmid)

Gel Extraction:

CRYAA Concentration
5.0ng/ul

Bba_K880005 Concentration
11.0ng/ul

Bba_K880005 Concentration

Conclusion:

Next step -> Ligation and Transformation

CRYAA and Bba_K880005 (Vector) Ligation and Transformation

Monday, June 06, 2016 4:33 PM

Goal: To back insert CRYAA to BBa

Procedure:
For Ligation Mixture

	Vol. in µl
Buffer	2
Enzyme	1
Vector	3.1
Insert	5
Water	8.9
Total	20

For Vector Control

	Vol. in µl
Buffer	2
Enzyme	1
Vector	3.1
Water	13.9
Total	20

For Transformation:

- Follow protocol (For Ligation Mixture, Vector Control, and Transformation Control)

Result:
Transformation was successful

Conclusion:
Move on to PCR check

BBa_K880005_CRYAA MiniPrep + Digestion (Bba_K880005_CRYAA + HisTerm) + Gel Run + Gel Extraction

Monday, June 13, 2016 10:53 PM

Goal: to remove from bacteria of CRYAA and cut at restriction site of both His_Term and Bba_K880005 _CRYAA to combine the two parts)

Procedure:

- Same as before

Results:
Conc of CRYAA: 120.0 micrograms/ liters

Conclusion:
Move on to Ligation

Bba_K880005_CRYAA and HisTag term Ligation and Transformation

Thursday, June 16, 2016 10:07 AM

Goal: Combine His tag Term with CRYAA

Procedure: same as before

Results:

Ligation:

Concentration of Histag Term (Vector): 15.9 <2200bp>
Concentration of CRYAA (Insert): 5.4 <522bp>

Buffer: 2
Enzyme: 1
Vector: 2.4
Insert: 5
Water: 9.6
Total: 20

Transformation:

- 3 colonies
    - Ligation, vector, transformation control

Conclusion: Transformation was successful

Bba_CRYAA Histerm PCR Gel Check & 3 in 1

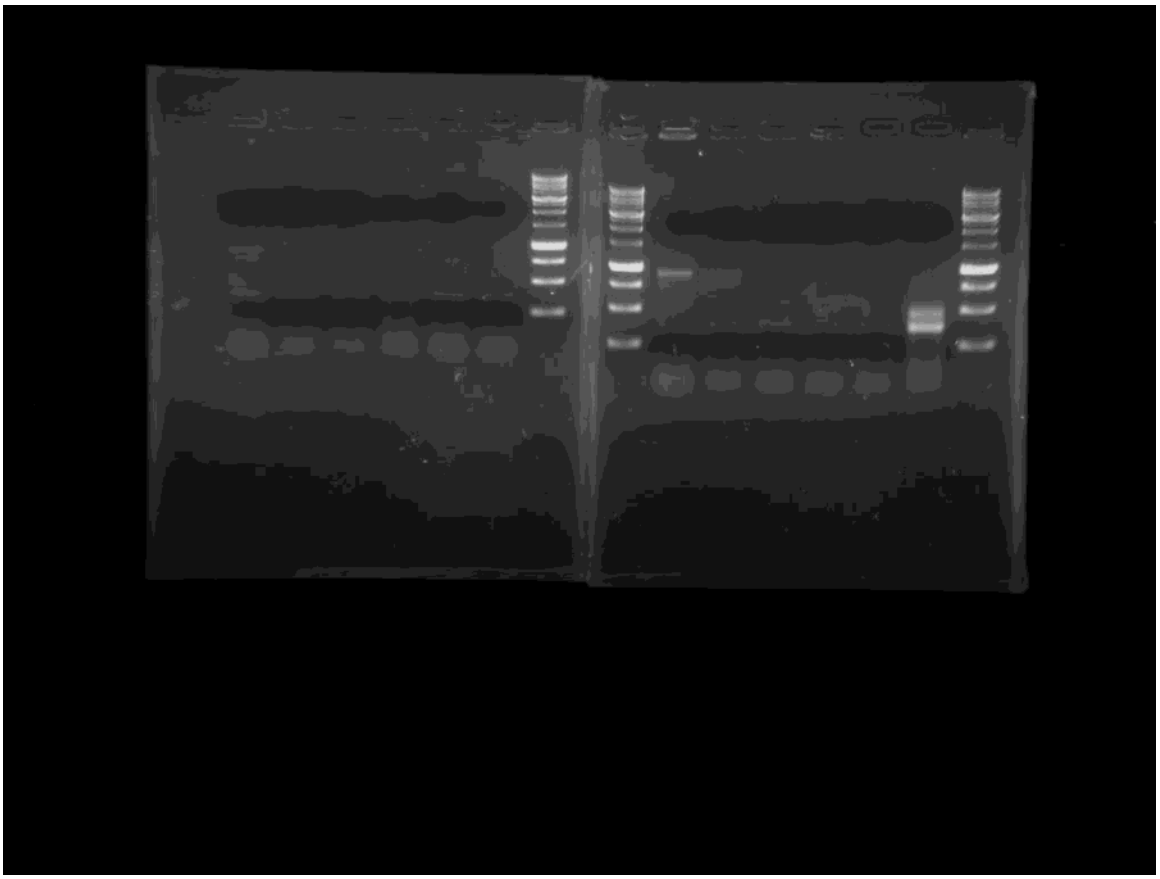
Friday, June 17, 2016 3:37 PM

Goal: PCR check and run gel to check if the construct was successfully cloned.

Procedure: Same as before

- 12 PCR tubes

Results:



Column: 1, 7, 8 succeeded

Conclusion:

Need to be sent out for sequencing to fully check whether the construct contains all parts of our composite part.

Bba_CRYAA Histag Term Liquid Culture

2016年6月17日　　下午 04:03

Goal: To later miniprep to send the construct for sequencing.

Conclusion:
Next Step -> Mini Prep for sequencing

Cloning Cycle Redo

Wednesday, June 29, 2016 12:19 PM

All the previous cloning cycle including Bba_K880005 and His Tag Term were all shut down because the sequencing revealed that we did not have both part.

GSR in iGEM Backbone/Vector was correct.

Shut down Bba_K880005_CRYAA_His Term construction

Tuesday, October 18, 2016 6:44 PM

The sequencing result was incorrect.

The purpose of CRYAA along with CRYAB was to model the aggregation of crystalline proteins in our fish lens cataract model but since too much resource and time was spent on both CRYAA and CRYAB, we decided to focus on CRYAB which was completed and corrected through mutagenesis by Mission bio tech during July in NYMU lab while TAS lab was closed.

CRYAA construct was eliminated from our project.